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EXHIBIT A

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Subcloning p11 (pT1-HP): the Hind III-PstI
0.95kb Fragment of Genomic Clone T11

870409

Subcloning

p11

Sac I - Pst I
Pst I - Hind III

Digestion of p11-6 by Sac I - Hind III - Pst I

DNA 45 μ l ($\leq 3 \mu$ g) 1 μ g p11-6 (4/9 min prep.)Sac I 2 μ l10x B 20 μ lddH₂O 133 μ ltotal 200 μ l

37°C 1 hr

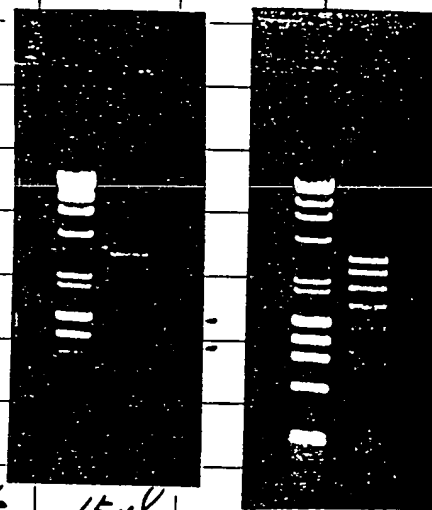
Hind III 2 μ l10x B 10 μ l1M Tris 8.0 8 μ l5M NaCl 2 μ lddH₂O 78 μ ltotal 300 μ l

37°C 1 hr

Pst I 2 μ l~~10x B 10 μ l~~5M NaCl 3 μ lddH₂O 95 μ l

Sac-Pst I 1.2 kb 80 ng

Pst I - Hind III 0.95 kb 63 ng

400 μ l 37°C 1 hr \rightarrow check 15 μ l5 μ l (5 μ g tRNA)12 μ l (5M NaCl) \downarrow Phenol ext. 5 min. (\rightarrow GFAA ext.) \downarrow c/g 10 min. \downarrow EtOH 1 ml dry ice. 20 min. \rightarrow c/g 15 min \downarrow Wash c 70% EtOH \rightarrow c/g 5 min \downarrow lyophilize 7 min \downarrow Resolue in 32 μ l TE + Bt 8 μ l

870409

Subcloning

p11 / SacI-HindIII 2.2 kb fragment

(cont.)

resolve in 50 μ l TE

Add Sph B-J

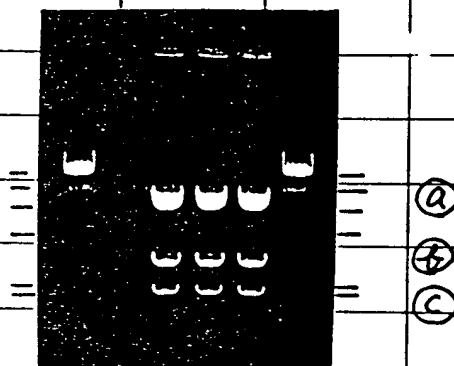
EP 1% Agarose

(Embryo gel 3 lanes)

214 0.5 μ g

Gene clean.

① Agarose 0.1 g

NAI 25 μ l 250 μ lGlass milk 5 μ lNEW 250 μ lTE 2.5 μ l \times 2

Subcloning

p11 / SacI-PstI 1.3 kb

inst

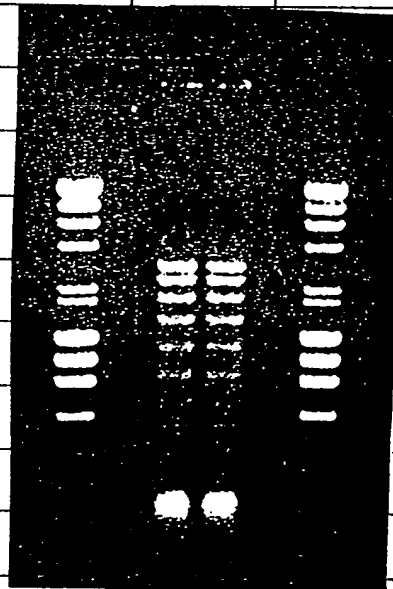
p11 / PstI-HindIII 0.95 kb

EP 1% Agarose

(Minigel 2 lanes)

Gene clean 1.2 0.95

Agarose 0.07 0.18

NAI 175 μ l 450Glass milk 5 μ l 5 μ lNEW 250 μ l 250 μ lTE 2.5 \times 2 2.5 \times 2

→ 25 ng
→ total 20 ng

Sequencing p11 (0.95kb)

870412

Sequencing of p11 - PstI HindIII / 0.95 kb

#128-2 18 µl. Alkali denature

(left primer (R-over)
right primer $16000 \text{ v/hr} / 1300 \text{ v} = 12.3 \text{ hr}$

1 20:45 →

2 22:35 → 10:55

Fixation 10% methanol / 10% Acetate / 2h
 15 min. after detaching gel from plate
 Gel dryer 80°C 50 min
 Autoradiography O/N RT

1

$$50\% - \frac{h}{m}$$

① $93 / 112$
 $= 83.0\%$

② $79 / 94$
 $= 84.0\%$

IFIND - INTELLIGENETICS

Page 1

→ exon

P11SA--MOUSE PDGF RECEPTOR

460

TTGGCTTTTAGTGGCACCCTTACCCCGGCATGATGGTGGATTCTACTTTCTACAATAA

CTTCACACTGGTGGCACCCTTACCCAGAGCTGCCCATGAACGACCAGTTCTACAATGC

ATLGGTTPPELPMDQFYNA

521K(S)K(R)M(K)P(D)H

GATCAAGAGTGGGTACGGATGCCAAGCCTGACCACGACTACCAAGTGAAG

CATCAAGAGGGGCTACCGCATGGCCCAGCCTGCTCATGCCTCCGACGAGATCTAT

IKRGYRMAQPAHASDETY

Score=38, Matched=63, Mismatched=27, Unmatched=1, Gaps=1
 Window=20, Word-size=2, Density=Less, Gap-Penalty=4

20
 30 60%

Ind. 101
GT-AG

Titration and Mini-Prep of the Okayama-Berg
cDNA Library (Normal Human Fibroblasts cDNA Library)

870629

Titration of D-B cDNA library (DHS)

 $9.5 \times 10^4 / \text{ml}$ $1 \times 10^4 / \mu\text{l}$ ($1 \times 10^9 / \text{ml}$)

DH1

 $1.0 \text{ OD}_{550} = 0.5 (\sim 5 \times 10^7 \text{ cells/ml})$ $10 \mu\text{l} \rightarrow 1 \text{ ml}$ $1 \times 10^4 / \mu\text{l}$ $10 \mu\text{l} \rightarrow 1 \text{ ml}$ $1 \times 10^2 / \mu\text{l}$ $10 \mu\text{l} \rightarrow 1 \text{ ml}$ $1 / \mu\text{l}$ $10 \mu\text{l}$ 10^3

x

111

 $1 \mu\text{l}$ 10^2

3

11

 $10 \mu\text{l}$ 10

2

 $1 \mu\text{l}$ 1

1

 $22.45' - 10.45' (12.45')$ $- 16.30' (18.15')$ Titer : $1.1 \times 10^5 / \mu\text{l}$

870630

 $1.5 \times 10^2 / \text{plate} \times 66 \text{ plate}$ $10 \mu\text{l} / \text{plate} \times 66 \text{ plates (660 } \mu\text{l)}$ $13.6 \mu\text{l}$ $1.5 \times 10^6 / 1000 \mu\text{l}$

870710

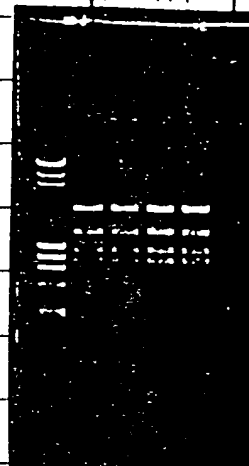
#248

Mini-prep. of O-B cDNA clone 1

O/N liquid cultures were stored in 15% glycerol at -20°C

#248

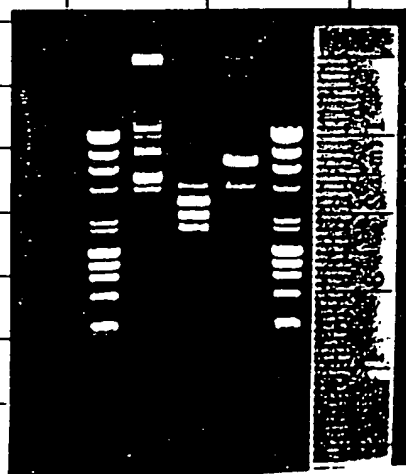
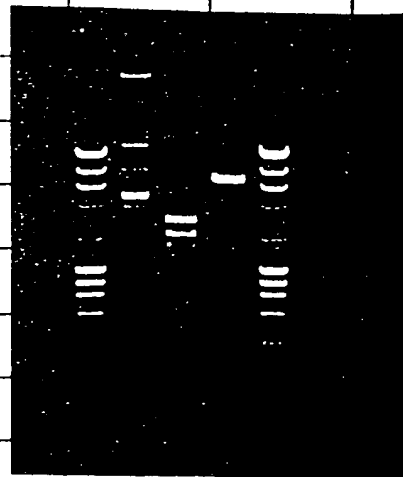
DNA	4 μ l (-1~4)
BamHI	1 μ l
10x B	1 μ l
ddH ₂ O	4 μ l
Total	10 μ l



OB vector 3.6
- 1.7
- 1.25
- 1.0
2.25 5.8 7.5 - 3.6 - 3.9 kb

DNA	4 μ l (1-1)
SalI or XhoI	1
10x B	1
ddH ₂ O	4
Total	10 μ l

Cont. XhoI SalI



8870711 M

Isolation of 0.2kbp Subfragment of λ EF 17 To Screen
a M426 Human Embryo Fibroblast cDNA Library

#420

880103

Isolation of EcoRI 0.2kb fragment from pMF17-2

#352 437 ng/μl pUC13

DNA 343 μl 150 μg

* $\frac{0.2}{2.7+0.2}$

EcoRI 75 μl

10×B 50 μl

RNase 10 μl

ddH₂O 22

total 500 μl

37°C 20 min

4% NuSieve gel

15 V const.

o/n

w/o circulation

EcoRI 0.2

T11

T11DEC

gel

0.83

1.93

1.76 g

NaI

2.1

4.9

4.4 ml

Gloss milk

15 μl

10 μl

10 μl

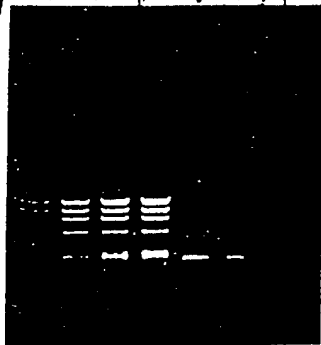
Save TE

14.5

9.5

9.5 μl

11.8 μg 0.25/0.5/2.25/1/1 μl 0.5 μl



#420 MF17-2 : 0.2kb fragment

80 ng/μl

880106

Nick translation to screen Toru's Library

#420 MF17-2 EcoRI 0.2kb (80ng/pl)

DNA 1.3 pl 100ng

SalI 10

II 5

32P 20

ddH₂O 13.7

total 50 pl

filter

20

HB

100 ml

SS DNA

400 pl

SAM NO

POS

TIME MIN

32P

CPM %ERROR

1 1-1

1.00 1258039.0 0.18

$$1.2 \times 10^6 \times 10^2 / 100 \text{ ng} \\ (1.15 \times 10^9 / \text{ng})$$

Mini-Preparation of Plasmid DNA: DNA Clones TR1
through 6 and TR8

#12 - #448

pEV7/TR1-8

880115

Preparation of plasmid DNA pEV7 TR1-8.

w/o RNase

DNA	5 μ l	5 μ l
BamHI	1 μ l	SacI 1 μ l
10 \times IB	1.5 μ l	10 \times IB 1.5
RNase	1 μ l	1
ddH ₂ O	6.5 μ l	6.5
total	15 μ l	15

Control

SVB/TII-A/ BamHI

SVB/BamHI XhoI

Control

SVB/TII-B/SacI #421 78ng/ λ

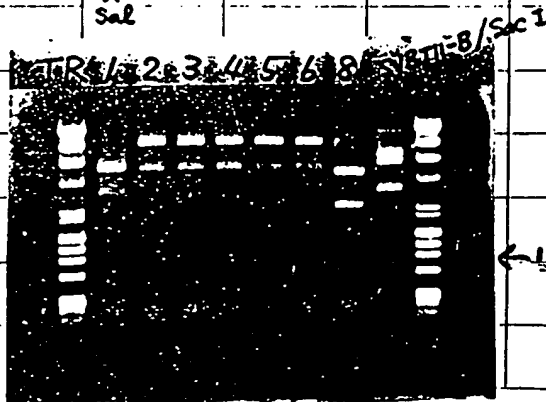
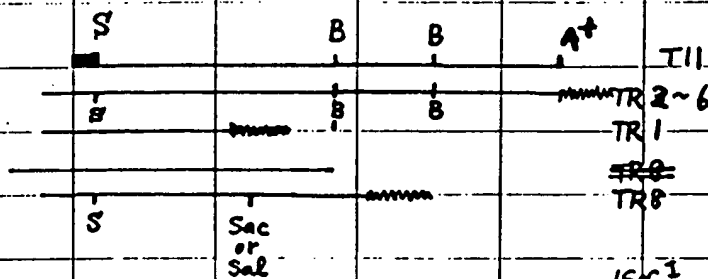
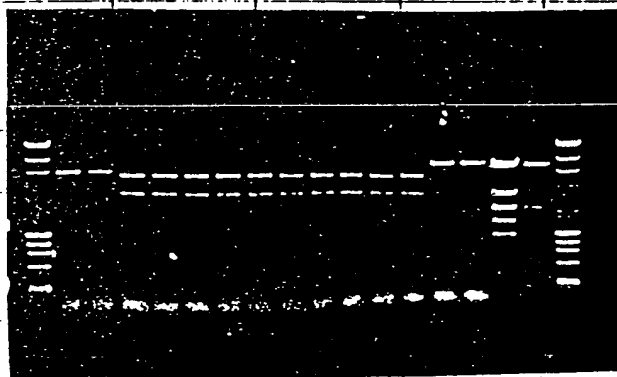
3.2, 0.3, 7.3, (6.5) kb

37°C 30min

tube

SalI 1

BamHI



Vector SacI 0.7kb

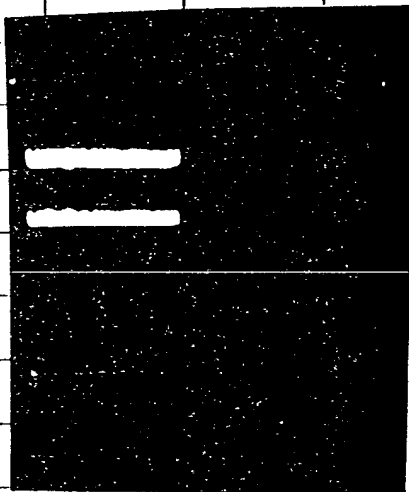
1/29 RNase (+)

Subcloning TR4

880216

Subcloning		TR4 BamHI	3.4kb fragment	into	SVX LTR2		
	#452	pCEV9/TR4	360 μ g/ml	pSVX 3032N	253 μ g/ml		
DNA	56 μ l	20 μ g		DNA	79	20 μ g	
BamHI	8	25 μ l		BamHI	8		
10x B	30			10x B	40		
ddH ₂ O	206 μ l			ddH ₂ O	273		
Total	300 μ l	check 8 μ l		Total	400	37°C 1hr	check 8 μ l
		0.8% Agarose	O/N		phenol ext.		

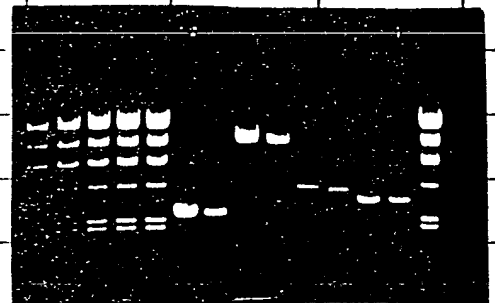
cut →



TR4 SVX (BamHI)



see Reverse side



glass milk	15 μ l			15:00 - (3hr)					
Ligation	Reaction								
Vector	SVX/BamHI #457	0.8	LTR2/BamHI #950 (100ng)	0.5	pUC18/SmaI 25ng 0.5	pUC18/SmaI 25ng 0.5			
Insert	TR4 BamHI #458 (100ng)	2	TR4 BamHI #458 (100ng)	2	#470X6-N-L 32ng 3.2	#470X6-N-S 32ng 3.2			
T4 ligase		1		1					
5x B		2		2					
ddH ₂ O		4.2		4.5					
total		10		10					
		1 μ l		1 μ l					
For transformation of JM101		1/10 μ l		1/10 μ l		2/10		3/10	

Minipreparation of Plasmid DNA: pSSV/TR4 (α -PDGF-R)
and HPR (β -PDGF-R)

880325

557 # 556

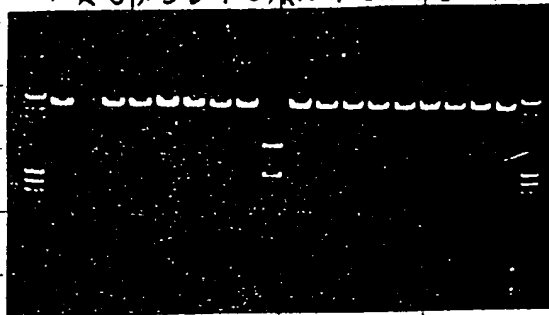
Miniprep of Plasmid DNA pSSV/TR4 or HPR

#557
HPR#556
TR4DNA 5 μ lSalI 2 μ l10 \times B 1.5ddH₂O 5.5

RNase 1

15

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

DNA 5 μ lXbaI 2 μ l10 \times B 1.5ddH₂O 5.5

RNase 1

15

#556 pSSV/TR4 SalI-RsaI(Xba)

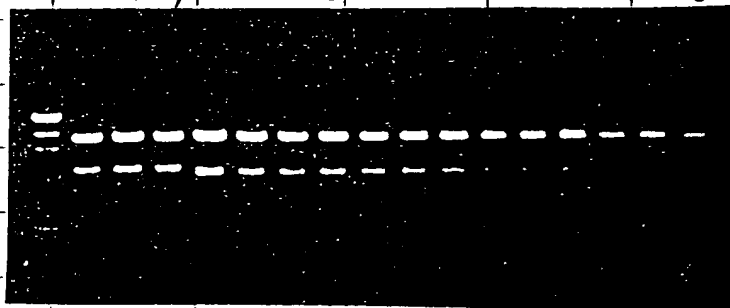
#557 pSSV/HPR SalI-Nhe(Xba)

HPR

TR4

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

SalI 2

10 \times B 1.318.3 μ l

For large prep. (1l)

4

7

Binding of ^{125}I -labeled Human PDGF to Control Mouse
3T3 cells, Control COS-1 cells and COS-1 cells
transfected with T11 (α -PDGF-R) HPR
(β -PDGF-R) cDNA Expression Vectors

^{125}I - PDGF binding

32D

8 wells + 4 wells

32D-HPR

8 wells + 4 wells

^{125}I - C-SIS

0.5 μl / well

8 μl / total 8 ml

C-SIS

250 ng / μl 100%

2 μl / 8 wells

A-A

210 ng / μl 75%

2 μl / 8 wells

huPDGF

Lot 68-1198
242 ng / 0.8 μl

12.9 μl / 8 wells

w/o competition

^{125}I - huPDGF

0.6 μl / well (1 ng)

4.8 μl / total 4 ml

w/o competition

huPDGF

~~250 ng / 0.8 μl~~
242 ng / 0.8 μl

(Control
2HAcetate
12.9 μl)

12.9 μl / 8 wells

1. Fibronectin coating

Inoculate
(30 min.)

2 x 12 wells / 100 μg

2. Cells

a)

32D J64

85 ml / flask

b)

16.2 HPR

cfu & resuspended in 50 ml DHEM

cfu & " 48 ml DHEM

Plate 2 ml / [fibronectin coated 12 wells plate] well

& Inoculate 30 min at 37°C

3 Wash the cells & take off the non-adherent cells

by using Binding Buffer (DHEM + 25 mM HEPES
1 mg/ml BSA)

4 Binding

RT

1 hr

Washing by 2 ml Binding Buffer x 4 times

5 Add 200 μl Solubilizing Buffer & sit RT 30 min

6 Count

880328

1	32D	¹²⁵ I - C-sis	-	306	
2			-	258	282 ± 34
3			C-sis	256	
4			"	260	258 ± 3
5			A-A	297	
6			"	244	297 ± 37
7			HuPDGF	329	
8			"	264	297 ± 46
9		¹²⁵ I - HuPDGF	-	682	
10			-	794	738 ± 79
11			HuPDGF	506	
12			"	527	517 ± 15
13	32D/HPR	¹²⁵ I - C-sis	-	2014	
14			-	1979	1997 ± 25
15			C-sis	331	
16			"	324	328 ± 5
17			A-A	1950	
18			"	2046	1998 ± 68
19			HuPDGF	902	
20			"	960	931 ± 41
21		¹²⁵ I - HuPDGF	-	850	
22			-	634	742 ± 153
23			HuPDGF	608	
24			"	544	576 ± 45
		¹²⁵ I - C-sis	20 μ l	943	total/well 690 μ l 23103
		¹²⁵ I - HuPDGF	20 μ l	1291	total/well 690 μ l 31630

Christy

